DIURNAL MOVEMENTS OF THE EYE PIGMENTS OF ANCHISTIOIDES

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I

Physiological rhythms which have been established in organisms by regular environmental changes, such as the change in light intensity between day and night, occasionally persist when the external recurring stimuli are removed and the organism is kept under constant conditions. An example of such a phenomenon is seen in the movements of the eye pigments of certain crustaceans. The distal pigment cells in the eye of *Macrobrachium* continue to move back and forth along the ommatidia when animals are kept under constant illumination (Welsh, 1930*b*). The proximal pigment in the eye of *Cambarus* shows a partial migration, with periods corresponding with day and night, when animals are kept in constant darkness (Bennitt, 1932). Several species of Bermuda crustaceans show diurnal changes in one or more of the three sets of pigments of the eye when kept in light of a constant intensity or in constant darkness (Welsh, 1935).

When regularly recurring variations in external conditions are prevented, as they may readily be in the case of aquatic organisms, and the organism still responds as though in its normal environment, it is possible to account for the response only on the basis of an internal controlling mechanism. In the case of a diurnal rhythm the response is normally due to changing light intensity, temperature, humidity, or some other factor which varies with day and night. The response might be due entirely to the direct effect of one or more of these factors but if it persists under constant conditions it is evident that it is more complex than this and is probably effected by way of the nervous system or a combination of nervous and endocrine systems.

It would appear to be of rather general interest to have more detailed information on the nature of the controlling mechanism and the crustacean material is quite satisfactory for such an investigation. One fact that must be known in the case of the changes in the crustacean eye is how long the rhythm persists. The present paper is concerned primarily with this problem.

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II

In 1933 two specimens of an unusual shrimp were taken in Bermuda by the author while collecting at night. In 1934 Dr. F. A. Brown obtained many more specimens which eventually proved to be *Anchistioides antiguensis* Schmidt, a form rarely taken in the Atlantic Ocean. Brown and Wheeler have been able to show (results not yet published) that this form is active only at night and can be collected in numbers only on the dark of the moon, thus showing a lunar periodicity. Because of its transparency, no pigment being present except in the eyes, it was selected for the present work. Preliminary observations on the eyes of animals kept in light of constant intensity or in constant darkness, except for short periods of examination, indicated slight diurnal changes in the distal pigment of the eye in the light, and complete normal daily movements in darkness. The appearance of such eyes when observed microscopically by means of transmitted light may be seen in Fig. 1, *A–D*. The condition seen in the eye of an animal kept in constant illumination and observed during the day will for convenience be called the " day-light " condition and may be seen in Fig. 1*A*. The same eye at night shows the distal boundary of the pigment nearer the periphery of the eye and this will be called the " night-light " condition (Fig. 1*B*). An animal removed from the dark during the daytime, and observed immediately, shows the distal pigment in the same position as is seen in the light during the day. This will be called a " day-dark " eye (Fig. 1*C*). If the same animal is returned to the dark and an examination made at night the entire eye is seen to be black except for a very thin border representing the cornea (Fig. 1*D*). This will be called the " night-dark " condition.

Observations of the intact eye show clearly the changes in position of the distal pigment cells and the changes which occur in the caps of reflecting pigment on the distal pigment cells, but changes in the main mass of reflecting pigment and in the proximal pigment can be seen only in sections of the eye.

The eye of *Anchistioides* as seen in sections is essentially like that of *Palæmonetes* which has been described by Parker (1897) and Welsh (1930*a*). The pigment changes are similar in most respects to those of *Palæmonetes* as described in these papers and may be compared with other crustaceans by reference to the review by Parker (1932).

Material for histological study was prepared in the following manner. A number of animals which had been caught the previous night were distributed among four containers. Two were illuminated with light from a 40W lamp at a distance of eighteen inches. Two were placed in a dark room. The period of adaptation began at 9:00 A.M. At 3:00 P.M. one lot was removed from the light and one from the dark and immediately fixed in hot water at 80° C. The heads were then cut off and placed in 70 per cent alcohol. At 11:00 P.M. the remaining two lots were treated in a similar manner. The eyes of a given lot of animals after sectioning showed the pigments in similar positions. Plate I, Fig. 3–6, show sketches of ommatidia based on camera lucida drawings. The situation is rather complex and each set of pigment must be fol-

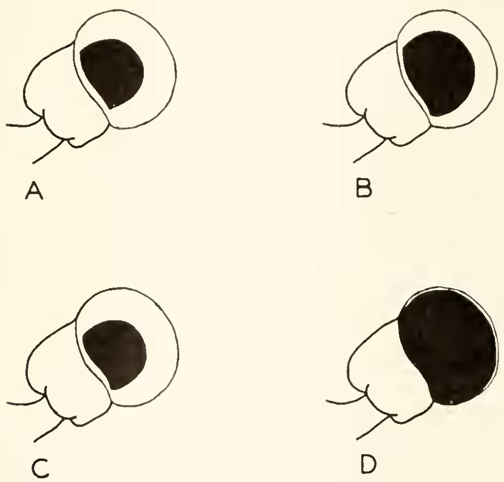

FIG. 1. *A*. Eye of an animal kept in constant illumination showing the position of the distal pigment cells during the day. A "day-light" eye.

B. Same eye as in *A* viewed at night. A "night-light" eye.

C. Eye of an animal kept in constant darkness except for short observational periods showing the position of the distal pigment cells during the day. A "day-dark" eye.

D. Same eye as in *C* viewed at night. A "night-dark" eye.

lowed through the series in order to make clear the changes which result from changes in light intensity and those which occur under constant conditions. The following summary supplements the figures and applies to the eye as a whole.

Day-light Eye. Fig. 3.

Distal Pigment Cells—In extreme proximal position resting on the retinular cells.

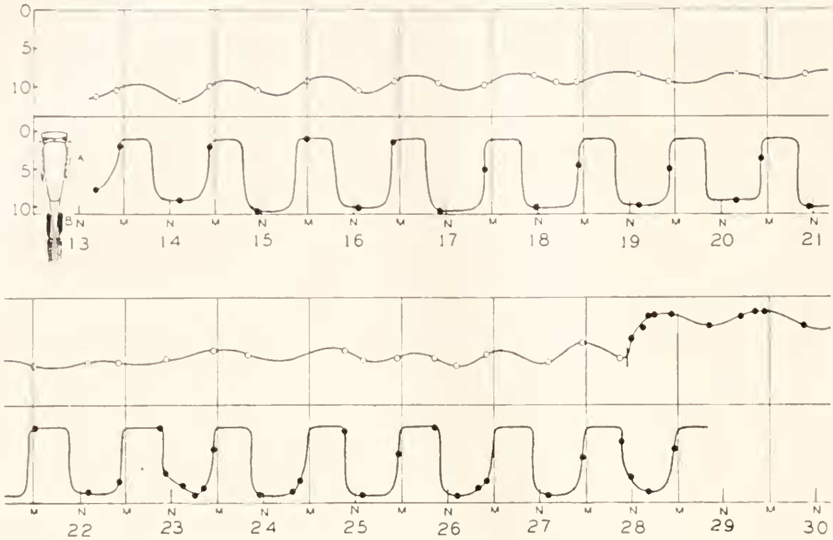

FIG. 2*A*. Records of the movements of the distal pigment cells of four animals kept in constant illumination (open circles), except for last two days, and of four animals kept in constant darkness (solid circles). Experiment started June 13 and ended on June 30. Noon and midnight are indicated by the letters *N* and *M*.

In the sketch of an ommatidium *A* is the extreme peripheral or night-position of the distal pigment cells, *B* the inner or day-position, in constant darkness.

The points as plotted are averages of the measurements of the distance from cornea to outer boundary of pigment. Each unit = 10 microns.

Time of sunset during course of the experiment about 7:35 P. M.

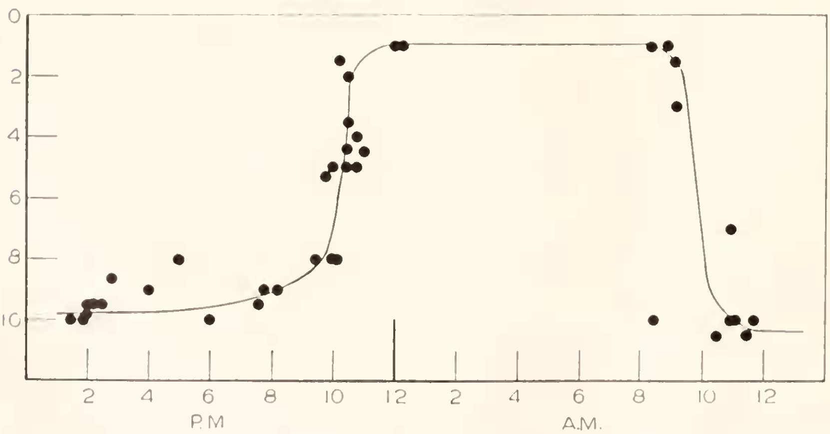

FIG. 2*B*. The measurements made on the eyes of animals kept in constant darkness and shown plotted over a period of fifteen days in Fig. 2*A* are here shown in one 24-hour period. Such a plot makes it possible to construct a curve representing the daily movements of the distal pigment cells. One of the coordinates shows the hours of the day beginning at noon, the other the distance of the distal pigment cells from the outer boundary of the cornea, each unit being equal to 10 microns.

Proximal Pigment—In retinular cells concentrated around rhabdomes. Some beneath the basement membrane.

Reflecting Pigment—Large masses resting on distal pigment cells, the remainder equally distributed above and below the basement membrane.

Night-light Eye. Fig. 4.

Distal Pigment Cells—Elongated but with proximal ends still in contact with retinular cells.

Proximal Pigment—Distributed as in day-light condition.

Reflecting Pigment—Most of this pigment which capped the distal pigment cells in the day-light conditions has moved proximally and fused with the mass below the basement membrane.

Day-dark Eye. Fig. 5.

Distal Pigment Cells—As in day-light condition.

Proximal Pigment—Above and below basement membrane. Very little in retinular cells.

Reflecting Pigment—Main mass around bases of retinular cells. Small caps on distal pigment cells and small masses below basement membrane.

Night-dark Eye. Fig. 6.

Distal Pigment Cells—In extreme distal position forming a collar around cones.

Proximal Pigment—Main mass below the basement membrane, none in distal part of retinular cells.

Reflecting Pigment—All is massed about the bases of the retinular cells where it forms a reflecting surface sending light back into the rhabdomes.

It is evident that the most striking changes in the eye which are independent of changes in the environment are seen when animals are kept in constant darkness. During the day the distal pigment cells are in the extreme inner position and during the night they move to the extreme outer position. In constant illumination, of the intensity used, the diurnal movement is not so striking as no change in position occurs, but only a change in length of the distal pigment cells.

It is not necessary to discuss further the changes in the reflecting pigment as they are clearly shown in the figures. The behavior of the reflecting pigment in certain other forms has been described elsewhere (Welsh, 1932). In this form the proximal pigment exhibits only slight changes which are independent of light and in this respect is like *Macrobrachium.*

In the case of *Macrobrachium* it had already been found (Welsh, 1930*b*) that the distal pigment cells continued their characteristic movements when the animals were constantly illuminated, and it was possible to follow closely the time relations and determine that the outward movement began at the time of sunset and the inward movement at the time of sunrise. Similar observations were made on *Anchistioides* kept in the light and in the dark. On June 13 four animals in separate bowls of sea water were placed in the dark and four were placed in a situation where they received only light from a 40W lamp. During the course of the experiment they were not fed but the water was changed once or twice each day. They were usually examined once near midday and once at about 11:00 P.M. The distance from the cornea to the periphery of the central pigment mass was then measured by means of an ocular micrometer. In the case of the animals kept in the dark this meant a period of illumination of a few minutes twice in twenty-four hours. Measurements were made for sixteen days when the experiment in the dark was discontinued as two of the animals died on June 29. On June 28 the animals which had been in the light for fifteen days were transferred to the dark and measurements continued for two days and then stopped as our stay in Bermuda was at an end.

The results are shown graphically in Fig. 2*A*. The points as plotted are averages of the measurements on the four animals. Although there were individual variations they were not great and each animal responded essentially as the group as a whole. In the dark the movement of pigment was as extensive at the end of the experiment as at the beginning, and the only change was a slight lag which may be seen by following the positions of the pigment at 10:30 or 11:00 P.M. when measurements were usually made. If all of the measurements made on the eyes of

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EXPLANATION OF PLATE I

Ommatidia (270 ×) showing details of pigment distribution.
Sketches are based on camera lucida drawings.

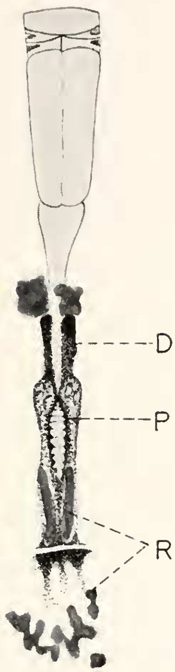
FIG. 3. Ommatidium from an eye of an animal constantly illuminated and killed and fixed during the day. "Day-light" condition.

FIG. 4. Ommatidium from an eye of an animal constantly illuminated and killed and fixed during the night. "Night-light" condition.

FIG. 5. Ommatidium from an eye of an animal kept in constant darkness and killed and fixed during the day. "Day-dark" condition.

FIG. 6. Ommatidium from an eye of an animal kept in constant darkness and killed and fixed during the night. "Night-dark" condition.

D = distal pigment; *P* = proximal pigment; *R* = reflecting pigment (the masses of reflecting pigment which rest on the distal pigment cells are not labelled).



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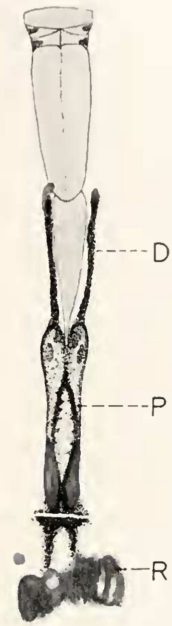

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Plate I

animals kept in constant darkness are plotted in one 24-hour period, as seen in Fig. 2*B*, it is possible to draw a curve which represents quite accurately the movements of the distal pigment cells. The outward movement of the pigment which begins at about the time of sunset is slower than the inward movement occurring in the morning. This is in agreement with the results obtained in a study of the rate of migration of the distal pigment cells in the eye of *Palæmonetes* (Welsh, 1930*a*), where it was found that distal migration took approximately twice as long as proximal migration. The curve obtained here may now be applied to the data as shown in Fig. 2*A* and justifies the curves which are shown in this figure.

As has already been pointed out, the changes in position of the distal pigment cells, in constant illumination of the intensity used in this instance was not great; nevertheless it is possible to follow their rhythmical migration, which, at least for a few days, kept in phase with day and night. On June 17 and 18 an apparent lag is noticeable and on June 20, 21, and 22 the pigment cells seem to be out of phase with day and night. During this period the changes in position of the pigment cells were very slight and it is quite possible that this is an incorrect interpretation as later on June 25, 26, and 27 they are again in phase. A rhythm approximating a 24-hour one was present, however, and continued throughout the experiment. Some change resulted from the prolonged exposure to light for when the animals were placed in the dark the movement was not as extensive as in those animals which had been kept in constant darkness. It is probable that longer stay in the dark would have resulted eventually in a return to a more normal condition. It is also possible, although the experiment was not tried, that constant illumination of a higher intensity than that used would have completely suppressed the movements of the distal pigment cells.

III

The persistence of a rhythm in the movements of the eye pigments of *Anchistioides* for a period of more than two weeks indicates that it is no transitory phenomenon with which we are dealing. The mechanism which is responsible for the changes in the eye continues its cyclic activity long after all recurring external stimuli are removed. It is highly unlikely that the rhythm is a property of the pigment cells. That it is a property of one of the main coördinating systems, either nervous or endocrine, is more nearly possible. Some of the evidence to support this conclusion will be considered briefly. In the earlier work on *Macrobrachium* it was suggested that both the nervous and endocrine systems were involved in the diurnal rhythm in the eye of this form. The results from the use of anaesthetics and the ligation of the eye-stalk led to this conclusion. The immediate control appeared to be hormonal yet somewhere in the chain the nervous system quite evidently played a part. At that time much less was known concerning the control of pigment migration in the crustacean eye than at the present time. Since then Kleinholz (1934, and in more recent unpublished work) has demonstrated, in a wide variety of decapod crustaceans, the part played by the so-called eye-stalk hormone in the activity of the eye pigments. It was reported (Welsh, 1935) that Kleinholz had found the eye-stalk extract controlled the inward movement of only the distal pigment cells. Since then the reflecting pigment has also been shown to be under the influence of material from the eye-stalk. No hormonal effect on the proximal pigment has yet been demonstrated. It is interesting to note that in *Anchistioides* the persisting movements are most extensive in the distal and reflecting pigments.

The work of Hanström (1931, 1934, 1935) and certain of his students (Sjögren, 1934; Carlson, 1935) has done much to further our knowledge of the sources of the eye-stalk hormone or hormones. These investigators have described two glands generally present in the crustacean eye-stalk which are quite certainly endocrine in nature. One they call the blood gland and the other the X-organ. Carlson has shown that the blood gland in *Uca* is the source of at least one of the pigment-concentrating substances affecting body chromatophores. The function of the X-organ still remains in doubt. These glands are supplied with nerves and their activities are doubtless controlled in part by impulses from the eyes arriving by way of the brain.

It was fortunately possible to have Dr. Hanström examine the eye-stalks of *Anchistioides* and he reported the presence of both the blood gland and the X-organ. The blood gland in this form is normally developed and is lying between the second and third optic ganglia on the dorsal side of the nerve mass. The X-organ is very well developed and is unusually far distal, almost touching the basal membrane of the eye. As in other decapods, its position is in the ventral part of the eye-stalk.

The hormone producing the movement, characteristic of the light, of the distal and reflecting pigments doubtless comes from one of these glands of the stalk. Normally we can assume that it is released into the blood stream under the stimulus of illumination, thence it is carried to the eye to produce so-called light adaptation of the eye. Under constant illumination in *Anchistioides* the production and secretion of the hormone continues and the pigment remains in a nearly constant light position depending on the intensity of illumination. In constant darkness, however, the situation is not so obvious. Why should the hormone be released during daylight hours and not during the hours of darkness and why should this process continue for two weeks without becoming irregular or showing signs of disappearing? At present it is only possible to speculate. There may be a rhythmic secretory cycle in the gland which continues under constant conditions or the situation may be much more complex and the rhythm in the eye may only accompany a general rhythmic activity which results from a series of changes involving the nervous-endocrine systems. Experiments which are now being carried on may throw further light on this interesting phenomenon.

Summary

1. The distal pigment cells and the reflecting pigment in the eyes of *Anchistioides*, a crustacean, continue their characteristic movements under constant external conditions.

2. The movements of the distal pigment cells continued for fifteen days in animals kept in constant darkness and in constant illumination. Movements, normal in extent, occurred in constant darkness while in constant illumination the movements were greatly suppressed.

3. The persistence of this diurnal rhythm indicates that an internal cyclical mechanism is responsible, in part, for pigment movements in crustacean eyes.

4. The possibility of the blood gland or X-organ being concerned in the diurnal rhythm in retinal pigment movement is considered.

LITERATURE CITED

Bennitt, R., 1932. Diurnal rhythm in the proximal pigment cells of the crayfish retina. *Physiol. Zoöl.*, **5**: 65.

Carlson, S. P., 1935. The color changes in Uca pugilator. *Proc. Nat. Acad. Sci.*, **21**: 549.

Hanström, B., 1931. Neue Untersuchungen über Sinnesorgane und Nervensystem der Crustaceen. I. *Zeitschr. f. Morph. u. Okol.*, **23**: 80.

Hanström, B., 1934. Über das Organ X, eine inkretorische Gehirndrüse der Crustacean. *Psychiatrische en Neurologische Bladen*, **38**: 405.

Hanström, B., 1935. Preliminary report on the probable connection between the blood gland and the chromatophore activator in decapod crustaceans. *Proc. Nat. Acad. Sci.*, **21**: 584.

Kleinholz, L. H., 1934. Eye-stalk hormone and the movement of distal retinal pigment in Palaemonetes. *Proc. Nat. Acad. Sci.*, **20**: 659.

Parker, G. H., 1897. Photomechanical changes in the retinal pigment cells of Palaemonetes, and their relation to the central nervous system. *Bull. Mus. Comp. Zoöl.*, **30**: 275.

Parker, G. H., 1932. The movements of the retinal pigment. *Ergebnisse der Biologie*, **9**: 239.

Sjogren, S., 1934. Die Blutdrüse und ihre Ausbildung bei den Decapoden. *Zool. Jahrbücher, Abt. f. Anat.*, **58**: 145.

WELSH, J. H., 1930*a*. The mechanics of migration of the distal pigment cells in the eyes of Palæmonetes. *Jour. Exper. Zoöl.*, **56**: 459.

WELSH, J. H., 1930*b*. Diurnal rhythm of the distal pigment cells in the eyes of certain crustaceans. *Proc. Nat. Acad. Sci.*, **16**: 386.

WELSH, J. H., 1932. The nature and movement of the reflecting pigment in the eyes of crustaceans. *Jour. Exper. Zoöl.*, **62**: 173.

WELSH, J. H., 1935. Further evidence of a diurnal rhythm in the movement of pigment cells in eyes of crustaceans. *Biol. Bull.*, **68**: 247.